IN THE SPECIFICATION

On page 12, line 28, insert the following:

--Description of Figures

FIG. 1:

Metabolism of ^{14}C-labeled PC into the neutral lipid fraction by plant microsomes. (A) Microsomes from developing seeds of sunflower, *R. communis* and *C. palaestina* were incubated for 80 mi at 30°C with PC (8 nmol) having oleic acid in its *sn*-1 position, and either ^{14}C-labeled oleic, ricinoleic or vernolic acid in its *sn*-2 position. Radioactivity incorporated in TAG (open bars), DAG (solid bars), and unsterified fatty acids (hatched bars) was quantified using thin layer chromatography followed by electronic autoradiography, and is shown as percentage of added labeled substrate. (B) Synthesis *in vitro* of TAG carrying two vernoloyl and one [^{14}C] ricinoleoyl group by microsomes from *R. communis*. The substrates added were unlabeled divernoloyl-DAG (5 nmol), together with either *sn*-1-oleoyl-*sn*-2-[^{14}C] ricinoleoyl-DAG (0.4 nmol, 770 dpm/nmol) or *sn*-1-oleoyl-*sn*-2-[^{14}C] ricinoleoyl-PC (0.4 nmol, 7700 dpm/nmol). The microsomes were incubated with the substrates for 30 min at 30°C, after which samples were removed for lipid analysis as described in the section "general methods". The data shown are the average of two experiments.

FIG. 2:

PDAT activity in yeast microsomes, as visualized by autoradiogram of neutral lipid products separated on TLC. Microsomal membranes (10 nmol of PC) from the wild type yeast strain FY1679 (lanes 1-3), a congenic yeast strain (FVKT004-04C(AL)) that is disrupted for YNR008w (lane 4) or the same disruption strain transformed with the plasmid pUS1, containing the YNR008w gene behind its native promoter (lane 5), were assayed for PDAT activity. As substrates, we used 2 nmol *sn*-1-oleoyl-*sn*-2-[^{14}C] ricinoleoyl-PC together with either 5 nmol of dioleoyl-DAG (lanes 2,4 and 5) or *rac*-oleoyl-vernoleoyl-DAG (lane 3). The enzymatic assay and lipid analysis was performed as described in Materials and Methods. The cells were precultured for 20 h in liquid YPD medium, harvested and re-suspended in an equal volume of minimal medium (19) containing 16 g/l glycerol. The cells were then grown for an additional 24 h prior to being harvested. Selection for the plasmid was maintained by growing the transformed cells in synthetic medium lacking uracil (18). Abbreviations: 1-OH-TAG, monoricinoleoyl-TAG; 1-OH-1-ep-TAG, monoricinoleoyl-monovernoloyl-TAG; OH-FA, unesterified ricinoleic acid.

FIG. 3:

Lipid content (A, B) and PDAT activity (C) in PDAT overexpressing yeast cells. The PDAT gene in the plasmid pUS4 was overexpressed from the galactose-induced

GAL1-TPK2 promoter in the wild type strain W303-1A (7). Its expression was induced after (A) 2 hours or (B) 25 hours of growth by the addition of 2% final concentration (w/v) of galactose. The cells were then incubated for another 22 hours before being harvested. The amount of lipids of the harvested cells was determined by GLC-analysis of its fatty acid contents and is presented as μmol fatty acids per mg dry weight in either TAG (open bar), polar lipids (hatched bar), sterol esters (solid bar) and other lipids (striped bar). The data shown are the mean values of results with three independent yeast cultures. (C) *In vitro* synthesis of TAG by microsomes prepared from yeast cells containing either the empty vector (vector) or the PDAT plasmid (+PDAT). The cells were grown as in Fig. 3A. The substrate lipids dioleoyl-DAG(2.5nmol) and *sn*-1-oleoyl-*sn*-2-[^{14}C] ricinoleoyl-PC (2nmol) were added to aliquots of microsomes (10 nmol PC), which were then incubated for 10 min at 28°C. The amount of label incorporated into TAG was quantified by electronic autoradiography. The results shown are the mean values of to experiments .

FIG. 4.

Substrate specificity of yeast PDAT. The PDAT activity was assayed by incubating aliquots of lyophilized microsomes (10nmol PC) with substrate lipids at 30°C for 10 min (panel A) or 90 min (panel B). Unlabeled DAG (2.5 nmol) was used as substrates together with different labeled phospholipids, as shown in the figure. (A) *Sn*-

position specificity of yeast PDAT regarding the acyl donor substrate. Dioleoyl-DAG together with either *sn*-1-[^{14}C] oleoyl-*sn*-2-[^{14}C]oleoyl-PC (di-[^{14}C]-PC), *sn*-1-[^{14}C] oleoyl-*sn*-2-oleoyl-PC (*sn*-1-[^{14}C]-PC) or *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PC (*sn*-2-[^{14}C]-PC). (B) Specificity of yeast PDAT regarding phospholipid headgroup and o fthe acyl composition of the phospholipid as well as o fthe diacylglycerol. Dioleoyl-DAG together with either *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PC (oleoyl-PC), *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PE (oleoyl-PE), *sn*-1-oleoyl-*sn*-2-[^{14}C] ricinoleoyl-PC (ricinoleoyl-PC) or *sn*-1-oleoyl-*sn*-2-[^{14}C] vernoloyl-PC (vernoloyl-PC). In the experiments presented in the 2 bars to the far right, monoricinoleoyl-DAG (ricinoleoyl-DAG or mono-vernoloyl-DAG (vernoloyl-DAG) were used together with *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PC. The labeled that was incorporated into TAG (solid bars) and lyso-PC (LPC, open bars) was quantified by electronic autoradiography. The results shown are the mean values of two experiments. The microsomes used were from W303-1A cells overexpressing the PDAT gene from the *GAL1-TPK2* promoter, as described in Fig. 3. The expression was induced at early stationary phase and the cells were harvested after an additional 24 h.

TAB. 1:

<u>In vitro synthesis of triacylglycerols in microsomal preparations of developing castor bean.</u> Aliqouts of microsomes (20 nmol PC) were lyophilised and substrate lipids were added in benzene solution: (A) 0.4 nmol [^{14}C]-DAG (7760dpm/nmol) and where

indicated 1.6 nmol unlabeled DAG; (B) 0.4 nmol [^{14}C]-DAG (7760 dpm/nmol) and 5 nmol unlabeled di-ricinoleoyl-PC and (C) 0.25 nmol [^{14}C]-PC (4000 dpm/nmol) and 5 nmol unlabeled DAG. The benzene was evaporated by N_2 and 0.1 ml of 50 mM potassium phosphate was added, thoroughly mixed and incubated at 30°C for (A) 20 min.; (B) and (C) 30 min. Assays were terminated by extraction of the lipids in chloroform. The lipids were then separated by thin layer chromatography on silica gel 60 plates (Merck; Darmstadt, Germany) in hexan/diethlether/acetic 35:70:1.5. The radioactive lipids were visualized and the radioactivity quantified on the plate by electronic autoradiography (Instant Imager, Packard, US). Results are presented as mean values of two experiments.

Radioactivity in different triacylglycerols (TAG) species formed. Abbreviations used: 1-OH-, mono-ricinoleoyl-; 2-OH, di-ricinoleoyl-; 3-OH-, triricinoleoyl; 1-OH-1-ver-, mono-ricinoleoyl-monovernoleoyl-; 1-OH-2-ver-, mono-ricinoleoyl-divernoleoyl-. Radiolabeled DAG and PC were prepared enzymatically. The radiolabeled ricinoleoyl group is attached at the sn-2-position of the lipid and unlabeled oleoyl group at the sn-1-position. Unlabeled DAG with vernoleoyl -or ricinoleoyl chains were prepared by the action of TAG lipase (6) on oil of Euphorbia lagascae or Castor bean , respectively. Synthetic di-ricinoleoyl -PC was kindly provided from Metapontum Agribios (Italy).

TAB. 2:

Total fatty acids per mg of T2 seeds pooled from individual *Arabidopsis thaliana* plants transformed with yeast PDAT gene under the control of napin promoter (26-14) or transformed with empty vector (32-4).

* = statistical difference between control plants and PDAT transformed plants in a mean difference two-sided test at $\alpha = 5$.

Description of the SEQ ID:

SEQ ID NO. 1: Genomic DNA sequence and suggested amino acid sequence of the *Saccharomyces cerevisiae* PDAT gene, YNR008w, with GenBank accession number Z71623 and Y13139, and with nucleotide ID number 1302481.

SEQ ID NO. 2: The amino acid sequence of the suggested open reading frame YNR008m from *Saccharomyces cerevisiae*.

SEQ ID NO. 3: Genomic DNA sequence of the *Schizosaccharomyces pombe* geneSPBC776.14.

SEQ ID NO. 4: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with GenBank accession number AB006704.

SEQ ID NO. 5: Nucleotide sequence of the *Arabidopsis thaliana* cDNA clone with GenBank accession number T04806, and nucleotide ID number 315966.

SEQ ID NO. 6: Predicted amino acid sequence of the *Arabidopsis thaliana* cDNA clone with GenBank accession number T04806.

SEQ ID NO. 7: Nucleotide and amino acid sequence of the *Zea mays* EST clone with GenBank accession number AI461339, and nucleotide ID number g4288167.

SEQ ID NO. 8: Predicted amino acid sequence of the *Zea mays* EST clone with GenBank accession number AI461339, and nucleotide ID number g4288167.

SEQ ID NO. 9: DNA sequence of part of the *Neurospora crassa* EST clone W07G1, with GenBank accession number AI398644, and nucleotide ID number g4241729.

SEQ ID NO. 10: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with GenBank accession number AC004557.

SEQ ID NO. 11: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with GenBank accession number AC003027.

SEQ ID NO. 12: DNA sequence of part of the *Lycopersicon esculentum* cDNA clone with GenBank accession number AI486635.

SEQ ID NO. 13: Amino acid sequence of the *Schizosaccharomyces pombe* putative open reading frame CAA22887 of the *Schizosaccharomyces pombe* gene SPBC776.14.

SEQ ID NO. 14: Amino acid sequence of the *Arabidopsis thaliana* putative open reading frame AAC80628 derived from the *Arabidopsis thaliana* locus with GenBank accession number AC004557.

SEQ ID NO. 15: Amino acid sequence of the *Arabidopsis thaliana* putative open reading frame AAD10668 derived from the *Arabidopsis thaliana* locus with GenBank accession number AC003027.

Further provisional and/or partial sequences are defined through the following SEQ Ids:

SEQ ID NO. 16: The amino acid sequence of the yeast ORF YNR008w from *Saccharomyces cerevisiae*.

SEQ ID NO. 17: Amino acid sequence of the region of the *Arabidopsis thaliana* genomic sequence (AC004557).

SEQ ID NO. 18: Amino acid sequence of the region of the *Arabidopsis thaliana* genomic sequence (AB006704).

SEQ ID NO. 19: The corresponding genomic DNA sequence and amino acid sequence of the yeast ORF YNROO8w from *Saccharomyces cerevisiae*.

SEQ ID NO. 20: The amino acid sequence of the yeast ORF YNR008w sequence and amino acid sequence of the yeast ORF YNR008w from *Saccharomyces cerevisiae* derived from the corresponding genomic DNA sequence.

SEQ ID NO. 21: Genomic DNA sequence of part of the *Saccharomyces cerevisiae* PDAT gene, YNR008w, genebank nucleotide ID number 1302481, and the suggested

YNR008w amino acid sequence.

SEQ ID NO. 22: The suggested amino acid sequence of the yeast gene YNR008w from *Saccharomyces cerevisiae.*

SEQ ID NO. 23: Genomic DNA sequence of part of the *Schizosaccharomyces* gene SPBC776.14.

SEQ ID NO. 24: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with genebank accession number AB006704.

SEQ ID NO. 25: Nucleotide sequence and the corresponding amino acid sequence of the *Arabidopsis thaliana* EST-clone with genebank accession number T04806, and ID number 315966.

SEQ ID NO. 26: Nucleotide and amino acid sequence of the *Zea mays* cDNA clone with genbank ID number g4388167.

SEQ ID NO. 27: Amino acid sequence of the *Zea mays* cDNA clone with genbank ID number g4388167.

SEQ ID NO. 28: DNA sequence of part of the *Neurospora crassa* cDNA clone W07G1, ID number g4241729.

SEQ ID NO. 29: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with genebank accession number AC004557.

SEQ ID NO. 29: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with genebank accession number AC003027.

SEQ ID NO. 30: DNA sequence of part of the *Lycopersicon esculentum* cDNA clone with genebank accession number AI486635.--

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